



**UNIVERSITI PUTRA MALAYSIA**

**QUALITATIVE AND QUANTITATIVE PCR METHODS FOR  
DETECTION OF FOODS CONTAINING GENETICALLY MODIFIED  
SOYBEAN AND CORN**

**TOSIAH ABDULLAH.**

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GENETICALLY MODIFIED SOYBEAN AND CORN**

**TOSIAH BT ABDULLAH**

**MASTER OF SCIENCE  
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**QUALITATIVE AND QUANTITATIVE PCR  
METHODS FOR DETECTION OF FOODS CONTAINING  
GENETICALLY MODIFIED SOYBEAN AND CORN**

**By**

**TOSIAH BT ABDULLAH**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Master of Science**

**March 2006**



## **DEDICATION**

**I wish to dedicate this work to my beloved Husband Mr. Abd Razak Kadri,  
my beloved children's, Noor Aida Shazwani, Muhammad Hakimi,  
Mohammad Sufi and Abdul Wafi, for their endless support to complete  
this study.**

**My beloved Son and My Father, Allahyarham Mohammad Iqbal and  
Allahyarham Hj. Abdullah b. Hj Bardan, ALFATIHAH,  
You are always in my heart,**

**My Mother Nairah Mukhsan, who always pray for me,**

**My brothers and my sisters,**

**My respected teachers and lecturers.**

**My dearest friends.**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirements for the degree of Master of Science

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METHODS FOR DETECTION OF FOODS CONTAINING  
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**TOSIAH BT ABDULLAH**

**March 2006**

**Chairman: Professor Son Radu, PhD**

**Faculty: Food Science and Technology**

Genetically modified organisms (GMOs) can be defined as organisms in which their genetic materials have been altered in the ways that does not occur naturally by mating or natural combination. Polymerase chain reaction (PCR) method is used to detect genetically modified events in foods. The specific objectives of this study are to establish a sensitive, robust and rapid method for the detection of genetically modified events by using PCR and to conduct a preliminary survey for distribution of foods derived from genetically modified events in Malaysia.

The two critical factors that were taken into account to achieve these objectives are the applicability of different DNA extraction methods for each kind of samples and PCR amplification conditions. Three different DNA extraction methods have been tested on soy, corn, potato and tofu (as a processed food).

The DNeasy method as in a widely used commercial kit, Wizard method (Hemmer, 1997) and Cetyl-trimethyl ammonium bromide (CTAB) method (Jankiewicz *et al.*, 1999) were evaluated in this study. The yield and purity of DNA were examined and compared. Quantification was accomplished by measuring UV absorbance at 260 nm and the suitability of DNA for PCR was tested. The results showed that there are significant differences between the methods used. CTAB, Wizard and DNeasy methods produced DNA with ratio of  $A_{260}/A_{280}$  range from 1.2 to 1.6, 1.9 to 2.2 and 1.7 to 1.9, respectively. However, the DNeasy method gave the optimum yield of DNA of high purity and was less time consuming. The primer pairs used for confirmation of the endogenous genes in the respective samples (*Lectin1* / *Lectin6* for *lectin gene* in soya, *Zein n-3'* / *Zein n-5'* for *zein gene* in maize and *Pss01 n-5'*/*Pss01 n-3'* for *patatain gene* in potato) produced the expected size of 318, 157 and 216 base pair, respectively.

The results of this study showed that 18 out of 85 soy samples were contaminated by at least one of three introduced genetic elements consisting 35S promoter, Nopaline Synthase terminator and the structural gene of 5-enolpyruvylshikimate-3-phosphate-synthase. Quantitative analysis of the 18 positive genetically modified soy samples showed that, seven samples contains 0.1 – 0.5% Roundup Ready Soy, four samples contains 0.5 – 1.0% Roundup Ready Soy and seven of them contains 1.0 – 2.0% Roundup Ready Soy.

In contrast, none of the 52 was positive with these assays. Therefore they were categorized as non-GM products.

These results revealed that PCR amplification method provides the key advantages of high sensitivity, robust and rapid operation whilst providing the requisites of careful experimental design that avoids both false-negative and/or false-positive results. Seven primer pairs (LEC1/LEC6; Zein n-3'/Zein n-5'; Pss01 n-5'/Pss01 n-3'; P35S 1-5'/P35S 2-3'; HA-NOS118-F/HA-NOS118-R, Cry1(A1)/Cry1(A2) and RRO1/RRO4) chosen in this study produced an expected size of 318, 157, 216, 101, 118, 107 and 356 base pair, respectively, fulfilling the product-size requirement and completed the whole detection procedure of GM events in food samples.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
dalam memenuhi keperluan untuk ijazah Master Sains

**PENGESANAN KUALITATIF DAN KUANTITATIF  
MENGUNAKAN KAEDAH TINDAKBALAS RANTAIAN POLIMER  
KEATAS MAKANAN BERASASKAN SOYA DAN JAGUNG YANG  
TERUBAHSUAI SECARA GENETIK**

Oleh

**TOSIAH BINTI ABDULLAH**

**Mac 2006**

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**Fakulti: Sains dan Teknologi Makanan**

Organisma terubahsuai genetik (GMO) boleh di definisikan sebagai organisma di mana pengubahsuaian kandungan genetikanya tidak berlaku secara kombinasi semulajadi. Kaedah tindakbalas rantaian polimerase (PCR) digunakan untuk mengecam GMOs dalam makanan. Objektif-objektif spesifik dalam kajian ini adalah untuk mengukuhkan kaedah operasi dalam pengecaman GMO dengan PCR yang sensitif, tegap and pantas serta mengendalikan pemeriksaan saringan terhadap pengagihan makanan terbitan dari GMOs di Malaysia.

Dua faktor kritikal yang diambilkira dalam mengecapi objektif-objektif tersebut adalah aplikasi kaedah ekstraksi DNA yang berlainan untuk setiap sampel dan keadaan amplifikasi PCR. Tiga kaedah ekstraksi DNA digunakan didalam kajian ini untuk melihat hasil dan kualiti daripada sampel soya,



jagung, kentang dan tauhu lembut iaitu kaedah Cetyl-trimethyl ammonium bromide (CTAB), Wizard dan DNeasy. Hasil and ketulenan DNA yang dihasilkan di analisa serta dibandingkan menggunakan pancaran Ultra violet pada jarak gelombang 260 nm dan menggunakan tindakbalas rangkaian polymerase.

Keputusan dari analisis perbandingan memaparkan bahawa terdapat perbezaan ketara bagi ketiga-tiga kaedah ekstraksi yang digunakan. Nisbah  $A_{260}/A_{280}$  bagi kaedah CTAB, Wizard dan DNeasy adalah antara 1.2 hingga 1.6, 1.9 hingga 2.2 dan 1.7 hingga 2.0, masing-masing. Walaubagaimanapun, kaedah DNeasy merupakan pilihan untuk kajian ini kerana kualiti DNANYa yang lebih baik dan masa analisa dapat dikurangkan. Tiga pasang primer khusus untuk pengesanan gen-gen kawalan bagi setiap sampel seperti gen lektin untuk soya, gen zein untuk jagung dan gen patatain untuk potato telah berjaya di amplifikasi dengan penghasilan amplikon bersaiz 318, 157 dan 216 pasangan bes masing-masing.

Keputusan tinjauan menunjukkan bahawa 18 daripada 85 sampel soya mengandungi kandungan genetik terubahsuai terdiri daripada sekurang-kurang satu daripada tiga unsur-unsur genetik iaitu '*35S promoter*', '*Nopaline Synthase terminator*' dan struktur gen '*5-enolpyruvylshikimate-3-phosphate-synthase*'. Pengesanan secara kuantitatif menunjukkan bahawa daripada 18 sampel soya tersebut, tujuh sampel mengandungi peratusan *Roundup Ready*

0.1-0.5%, empat sampel mengandungi peratusan *Roundup Ready* 0.5 -1.0% dan tujuh yang lain mengandungi peratusan *Roundup Ready* antara 1.0- 2.0%. Sebaliknya, tiada sampel jagung (52 sampel) adalah positif dengan analisis tersebut. Oleh yang demikian, sampel tersebut boleh dikategorikan sebagai produk bukan GM.

Keputusan kajian menunjukkan bahawa kaedah amplifikasi PCR membekalkan kunci kelebihan dari segi sensitiviti, ketegapan dan operasi yang pantas sejourus membekalkan keperluan dalam rekabentuk eksperimen yang teliti untuk mengelakkan keputusan negatif-tiruan dan positif-tiruan. Tujuh pasang primer, khasnya LEC1/LEC6; Zein n-3'/Zein n-5'; Pss01 n-5'/Pss01 n-3'; P35S 1-5'/P35S 2-3'; HA-NOS118-F/HA-NOS118-R, Cry1(A1)/Cry1(A2) dan RRO1/RRO4 yang dipilih dalam kajian ini menghasilkan amplikons dengan pasangan bes sebanyak 318, 157, 216, 101, 118, 107 dan 356 masing-masing telah memenuhi syarat saiz-produk pengesanan genetik terubahsuai didalam sampel makanan.

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I certify that an Examination Committee has met on 13 March 2006 to conduct the final examination of Tosiah bt Abdullah on her Master of Science thesis entitled "Qualitative and Quantitative PCR Methods for Detection of Foods Containing Genetically Modified Soybean and Corn" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



**TOSIAH BT ABDULLAH**

Date: 8/5/2006

## TABLE OF CONTENTS

	Page
<b>DEDICATION</b>	<b>ii</b>
<b>ABSTRACT</b>	<b>iii</b>
<b>ABSTRAK</b>	<b>vi</b>
<b>ACKNOWLEDGEMENTS</b>	<b>ix</b>
<b>APPROVAL</b>	<b>x</b>
<b>DECLARATION</b>	<b>xii</b>
<b>LIST OF TABLES</b>	<b>xvi</b>
<b>LIST OF FIGURES</b>	<b>xviii</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xxi</b>
<b>LIST OF SYMBOLS AND UNITS</b>	<b>xxiii</b>
 <b>CHAPTER</b>	
 <b>I INTRODUCTION</b>	 <b>1</b>
 <b>II LITERATURE REVIEW</b>	 <b>5</b>
Genetically Modified Food	5
Element of Gene Construct	6
Types of Genetic Modifications	8
The Novel food Regulations	8
Sampling of GM Products for Testing	10
Isolation and Purification of DNA, RNA or Protein	12
Extraction Methods	12
Pre-treatment	14
Lysis	14
Isolation and Purification Methods	15
References Material of GMO testing	20
Method for Detecting GMO in Food and it's Derivatives	21
Protein Based Testing Methods	22
DNA Based Testing Method	22
Polymerase Chain Reaction	23
Principles of PCR Method in GMO Analysis	24
Qualitative Detection	30
Quantitative Detection	32
Other Type of Methods	34
Advantages of DNA-based Compared with Protein-based for GMO analysis	36
PCR Target Sequences: Categories and Specificity	39
Category 1, Screening Methods	41
Category 2, Gene-specific Methods	41
Category 3, Construct-specific Methods	42

Category 4, Even-specific Methods	43
Polymerase Chain Reaction: Quality Assurance in GMO Analysis	44
<b>III COMPARATIVE EVALUATION OF THREE DIFFERENT DNA EXTRACTION METHOD IN FOOD SAMPLES</b>	<b>46</b>
Introduction	46
Materials and Methods	51
Food Samples	51
Sample Preparation	51
Method for DNA Extraction	51
DNeasy® Method	51
The Wizard Method	52
The CTAB Method	53
DNA Quantification	54
Primers	55
PCR Protocol	55
Agarose Gel Electrophoresis	57
Statistical Analysis	57
Results	58
Discussion	64
Evaluation on DNA Yields (ng DNA/mg sample) Of Three Plant DNA Extraction Methods on Four Categories of Samples	64
Evaluation on DNA Purity ( $A_{260}/A_{280}$ Ratio) Of three plant DNA extraction methods on four Categories of samples	66
Confirmatory Assay of DNA Quality Using PCR Amplification	68
<b>IV QUALITATIVE DETECTION OF GENETICALLY MODIFIED SOY IN PROCESSED FOOD BY POLYMERASE CHAIN REACTION</b>	<b>70</b>
Introduction	70
Materials and methods	72
Samples Collection	72
Sampel Preparation	72
Certified Reference Material	73
DNA Extraction	84
Oligonucleotide primers	84
PCR (Polymerase Chain Reaction)	85
Agarose Gel Electrophoresis	85
Results	86



	Discussion	97
<b>V</b>	<b>QUALITATIVE DETECTION OF GENETICALLY MODIFIED MAIZE IN PROCESSED FOOD BY POLYMERASE CHAIN REACTION</b>	<b>102</b>
	Introduction	102
	Materials and methods	104
	Samples Collection	104
	Sample Preparation	104
	Certified Reference Material	104
	DNA Extraction	110
	Primers	110
	PCR (Polymerase Chain Reaction)	111
	Agarose Gel Electrophoresis	114
	Results	115
	Discussion	120
<b>VI</b>	<b>QUANTITATIVE POLYMERASE CHAIN REACTION FOR THE DETECTION OF GENETICALLY MODIFIED FOODS</b>	<b>124</b>
	Introduction	124
	Materials and methods	128
	Samples	128
	Screening using Light Cyclor	129
	Quantitative PCR	133
	LightCycler PCR for Quantification	134
	Standard curves	134
	Results	137
	Discussion	144
<b>VII</b>	<b>GENERAL DISCUSSION AND CONCLUSION</b>	<b>147</b>
	Conclusions	147
	Recommendations	149
	<b>REFERENCES</b>	<b>151</b>
	<b>APPENDIX</b>	<b>164</b>
	<b>BIODATA OF THE AUTHOR</b>	<b>165</b>

## LIST OF TABLES

Table	Page
2.1 Recommended size of laboratory sample in case of homogenous distribution of GM particles in the investigated lot (3500 particles) at 1% threshold for GMOs according to Swiss Food Manual	12
2.2 Procedures for DNA extraction and purification from food matrixes	19
2.3 Plant species and genetic elements included in the GeneScan™ GMO Chip	24
2.4 Detection of GMO derivatives grouped according to catogeries of specificity	40
3.1 Primers Used in this Study	55
3.2 Amplification condition for PCR assay of lectin genes	56
3.3 Amplification condition for PCR assay of zein genes	56
3.4 Amplification condition for PCR assay of patatain genes	57
3.5 The yield and purity of DNA extracted from three different extraction methods	59
3.6 Single analysis of DNA yield (ng DNA/mg sample) for each kind of samples on three extraction methods	60
3.7 Single analysis of DNA purity ( $A_{260}/A_{280}$ ) for each kind of samples on three extraction methods	60
4.1 Description of the analyzed soybean samples in this study	74
4.2 Gene constructs in RR soybean	83
4.3 Detection of lectin gene, 35S promoter, nos terminator and specific gene (RRO) in food samples containing soy product.	87
5.1 Description of the analyzed maize samples in this study	105
5.2 Primers Used in this Study	111

5.3	Amplification condition for PCR assay of zein genes	112
5.4	Amplification condition for PCR assay of 35S promoter	112
5.5	Amplification condition for PCR assay of <i>nos</i> terminator	113
5.6	Amplification condition for PCR assay of CP4EPSPS genes	113
5.7	Amplification condition for PCR assay of Cry genes	114
5.8	Detection of zein gene, 35S promoter, <i>nos</i> terminator, CP4EPSPS and Cry gene in food samples containing corn product.	116
6.1	Kit Contents of LightCycler® GMO Screening Kit (Cat. No. 3 267 199)	130
6.2	Master mix reaction for LightCycler® GMO Screening Kit	131
6.3	Cycling Program for the LightCycler® GMO Screening Kit	132
6.4	Primer and probes for lectin and RRSoybean	133
6.5	A master mix with the following reaction components was use for the amplification of each PCR for quantification	135
6.6	Quantity of DNA and dilutions of the standard curves	135
6.7	Cycling Program for the Soy Quantification	136
6.8	Representative standard curves (RRS and lectin specific) to calculate the ratio of GMO in the samples using Linght Cycler® Instruments.	142
6.9	Percentage of Roundup Ready Soy in Samples	143

## LIST OF FIGURES

Figures		Page
2.1	Sample preparation step in GMO analysis	13
2.2	Structure of genetic information	25
2.3	The steps in PCR and the exponential increase in the overall number of DNA copies synthesized.	28
2.4	Flow chart of PCR method for detection of GM events in foods	32
2.5	Classification of methods for GMO analysis	35
2.6	A schematic representation of a typical gene construct and four types of PCR-based assays showing increasing specificity ( <i>from top to bottom</i> ).	39
3.1	Representative genomic DNA from DNeasy extraction method	61
3.2	Representative genomic DNA from Wizard extraction method.	61
3.3	Representative genomic DNA from CTAB extraction method.	62
3.4	Representative of PCR product for <i>lectin gene</i> from soy sample	62
3.5	Representative of PCR product for zein gene from maize sample	63
3.6	Representative of PCR product for patatain gene from potato sample	63
3.7	Representative of PCR product for lectin gene from smooth tofu sample	64
4.1	Representative of PCR product for <i>lectin gene</i> obtained from seeds or raw soybean samples in 2% agarose gel	89
4.2	Representative of PCR product for <i>lectin gene</i> obtained from soy flour samples in 2% agarose gel.	89

4.3	Representative of PCR product for <i>lectin gene</i> obtained from tofu sample in 2% agarose gel.	90
4.4	Representative of PCR product for <i>lectin gene</i> obtained from fucuk sample in 2% agarose gel	90
4.5	Representative of PCR product for <i>lectin gene</i> obtained from tempe sample in 2% agarose gel	91
4.6	Representative of PCR product for <i>lectin gene</i> obtained from soy milk and soy sauce sample in 2% agarose gel	91
4.7	Agarose gel electrophoresis of PCR products for 35S promoter obtained from positive sample in 2% agarose gel	92
4.8	Agarose gel electrophoresis of PCR products for 35S promoter obtained from positive sample in 2% agarose gel	92
4.9	Agarose gel electrophoresis of PCR products for <i>nos</i> terminator obtained from positive sample in 2% agarose gel	93
4.10	Agarose gel electrophoresis of PCR products for <i>nos</i> terminator obtained from positive sample in 2% agarose gel.	93
4.11	Agarose gel electrophoresis of PCR products for <i>Roundup Ready specific-GMO (RRO)</i> obtained from positive sample in 2% agarose gel	94
4.12	Agarose gel electrophoresis of PCR products for <i>Roundup Ready specific-GMO (RRO)</i> obtained from positive sample in 2% agarose gel	94
4.13	Agarose gel electrophoresis of PCR products for <i>Roundup Ready specific-GMO (RRO)</i> obtained from positive sample in 2% agarose gel	95
4.14	Agarose gel electrophoresis of PCR products for <i>Roundup Ready specific-GMO (RRO)</i> obtained from positive sample in 2% agarose gel	95

4.15	Representative of PCR products for positive GM soy in 2% agarose gel	96
4.16	A schematic presentation a Roundup Ready soybean cassette: P- 35S-CTP	100
5.1	Representative agarose gel electrophoresis of PCR products zein gene obtained from maize-derived raw material and products for analysis of maize	118
5.2	Representative agarose gel electrophoresis of PCR products obtained from corn-derived raw material and products for analysis of corn positive P35S promoter and NOS terminator.	118
5.3	Representative agarose gel electrophoresis of PCR products of <i>EPSPS</i> obtained from maize-derived raw material and products for analysis of maize	119
5.4	Representative agarose gel electrophoresis of PCR products of <i>Cry</i> gene obtained from maize-derived raw material and products for analysis of maize	119
6.1	Competitive quantitative PCR	126
6.2	The automatic detection (and display) of PCR product of representative positive sample ( <i>P35S</i> and <i>NOS</i> terminator) throughout the amplification process using LightCycler® GMO Screening kit	138
6.3	The automatic detection (and display) of PCR product of representative positive sample and standards using hybridization probes and primer in LightCycler® Instruments	139
6.4	The automatic detection (and display) of PCR product dilution of representative lectin standards throughout the amplification process.	140
6.5	The automatic detection (and display) of PCR product dilution of representative Roundup Ready Soy (RRS) standards throughout the amplification process.	141

## LIST OF ABBREVIATIONS

AIA	Advanced Informed Approval
CaMV	Cauliflower mosaic virus
CRM	Certified References Material
CP4-EPSPS	enolpyruvylshikimate-3-phosphate synthase from <i>Agrobacterium</i> sp.strainCP4)
CRD	Completed Randomize Design
Ct	Crossing Threshold
CTAB	Cetyl-trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
FRET	Flouresence Resonance energy Trasfer
E	Efficiency
EC	European Council
EDTA	Ethylene-diamine-tetra acetic acid
ELISA	Enzyme Linked Immunosorbant Assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
EtBr	Ethidium bromide
EU	European Union
FAO	Food and Agriculture Organisation
FDA	Food and Drug Association
GIPSA	Grain Inspection, Parkers and Stockyards Administration
GM	Genetically modified
GMAC	Genetically Modification Advisory Committee
GMF	Genetically modified food

GMO	Genetically modified organism
GMOs	Genetically modified organisms
HCL	Hydrogen Chloride
Mg	Magnesium
LFS	Lateral Flow Strip
MgCl <sub>2</sub>	Magnesium Chloride
MM	Maximizer maize
NA	Nucleic Acid
NaCl	Natrium Chloride
NaOH	Natrium Hydroxide
NIRS	Near-infrared spectroscopy
NOS	Nopaline Synthase
P35S	35S promoter
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PVP	Polyvinylpyrrolidone
QC-PCR	Qualitative Competitive PCR
RNA	Ribonucleic acid
RNase	Ribonuclease
ROSE	Rapid-One-Step-Extraction Solution
RRO	Roundup Ready Oligonucleotide
RRS	Roundup Ready soybean
SDS	Sodium dodecyl sulfate
TBE	Tris-Boric acid-EDTA buffer
UV	Ultraviolet
WHO	World Health Organization



## LIST OF SYMBOLS AND UNITS

bp	Base pair
$\beta$	Beta
$^{\circ}\text{C}$	Degree Celsius
U	Enzyme unit
kb	Kilo-base pair
$\mu\text{g}$	Microgram
$\mu\text{L}$	Microliter
mg	Milligram
mL	Milliliter
mM	Millimolar
M	Molar
ng	Nanogram
OD	Optical density
%	Percent
pmol	Picomole
rpm	Revolutions per minute
vol	Volume
v/v	Volume per volume
w/v	Weight per volume